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The development of efficient routes to carbohydrates in the form of glycolipids and glycopeptides has been our goal. Since these entities mimic components of the surface of tumor cells, the ultimate purpose of the research is to develop vaccines based on these glycolipids and glycopeptides. The report herein focuses on the initial phase of the synthesis of a mucin related *O*-linked glycopeptide consisting of Tn, TF, and Le^y antigens. TF and Tn are quite common in carcinoma malignancies, particularly of the colon and prostate while Le^y is over-expressed on many human tumor cells including those found in colon, lung, breast, and ovarian cancers.

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Marsha C Lawson 4-19-00

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Efforts toward Synthesis of a Le^y–TF–TN Glycopeptide: Recent Developments in Anti-tumor Glycopeptide-Based Vaccines

INTRODUCTION

The development of efficient routes for the preparation of complex oligosaccharide or carbohydrate conjugates has been a goal in the Danishefsky group for some time.[1] Synthetic investigations in this area can help to provide a detailed knowledge of the structural and chemical behavior of carbohydrates and their conjugates. Moreover, it has been known for some time now that specific types of glycolipids or glycoproteins, which are chemically detectable in normal cells, are more highly expressed in tumors. It should be noted that abnormally high levels of expression on tumor cells cause an antibody response, consequently rendering the cell-surface glycoconjugate a tumor-associated antigen. The idea of such glycoconjugates as tumor-associated antigens is the basis for using carbohydrates in the development of antitumor vaccines.[2] Since tumor antigens and vaccine constructs are usually inaccessible from natural sources, it falls to the organic chemist to supply necessary quantities of carbohydrates, in the form of both glycolipids and glycopeptides.

Progress Report **Matthew W. Carson**

Efforts toward Synthesis of a Le^y–TF–Tn Glycopeptide: Recent Developments in Anti-tumor Glycopeptide-Based Vaccines

Mucins, which comprise a family of large glycoproteins expressed on cells of epithelial tissues, carry large glycodomains in clustered modes.[3] Mucin amino acid sequences possess a very high percentage of serine and threonine residues, often found in contiguous arrays ranging in number from two to five.[4] *O*-linked glycopeptides of Thomsen-Friedenreich disaccharide (TF)[5] and Tn, as well as the blood determinant Lewis Y (Le^y),[6a–d] have been immunocharacterized as being over-expressed at the surface of malignant cells in a variety of cancers. With this in mind, the Danishefsky group set out to fashion vaccines based on the protein forms of these antigens (Scheme 1).

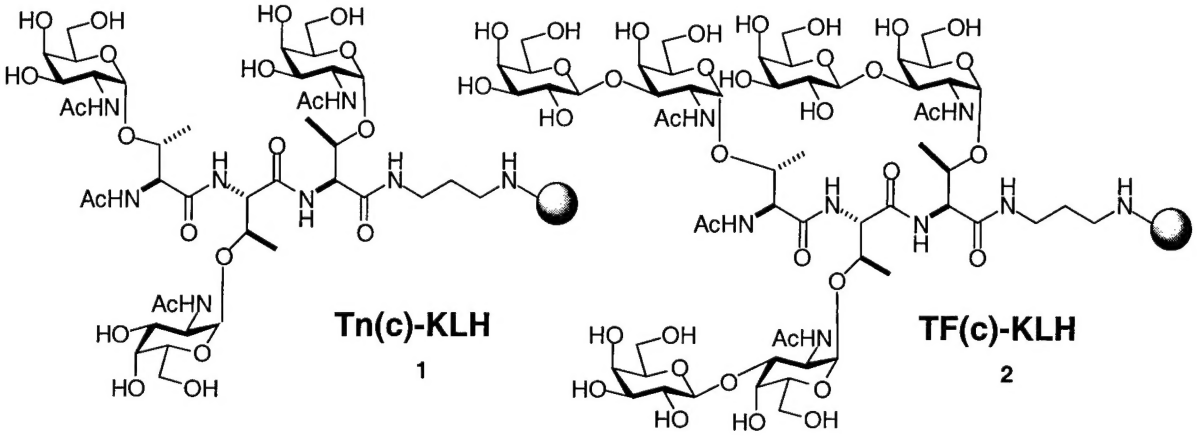
TF and Tn are quite common in carcinoma malignancies, particularly of the colon and prostate. As described by Dr. Scott Kuduk[7] in the previous progress report, the dissacharide TF, was synthesized using standard glycal assembly techniques. Attachment of a diamine linker to the TF antigen building block, followed by glycosylation of a threonine peptide chain via cassette methodology, yielded a clustered antigen motif. The resulting *O*-linked glycopeptide (**1**) was then attached to the protein carrier keyhole limpet hemocyanin (KLH), therefore rendering the glycopeptide immunogenic. The clustered mucin-related structure of Tn (**2**) was assembled in a similar fashion.

The mucin glycopeptide form of the antigenic blood group determinant Le^y (**3**) is over-expressed on many human tumor cells including those found in colon, lung, breast, and ovarian cancers.[8] A mucin mimic of Le^y, as illustrated in Scheme 1, was prepared and to our delight, preclinical evaluation demonstrated that the polypeptide-based vaccine provokes an anitbody response targeting Le^y –mucin bearing cells.[6,9] Clinical evaluation of an anti-cancer vaccine based on **3** is currently underway.

One of the current goals in the Danishefsky group is to synthesize a clustered antigen motif containing Le^y, TF, *and* Tn (Figure 1, **4**), containing a serine backbone and diamino linker. An efficient synthesis of the glycopeptide could lead to the development of a polyvalent vaccine. The possible advantages of this type of vaccine are: (1) a vaccine requiring multi anti-tumor activity could be prepared by synthesizing just one glycopeptide and; (2) three different antigens in one clustered motif could result in a synergistic effect regarding anti-tumor activity. Reported herein is the initial phase of the synthesis of a Le^y, TF, *and* Tn glycopeptide mucin mimic (**4**).

Scheme 1

Mucin Cluster Vaccines

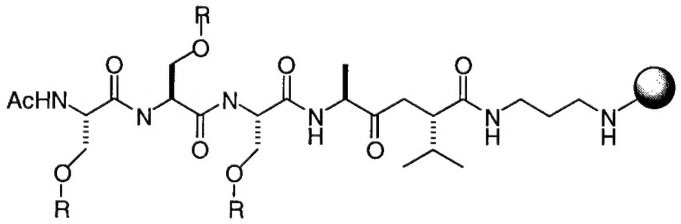


Tn(c)-KLH

1

TF(c)-KLH

2



Le^y(c)–KLH

3

R =

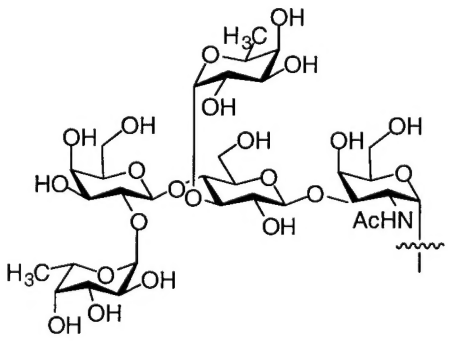
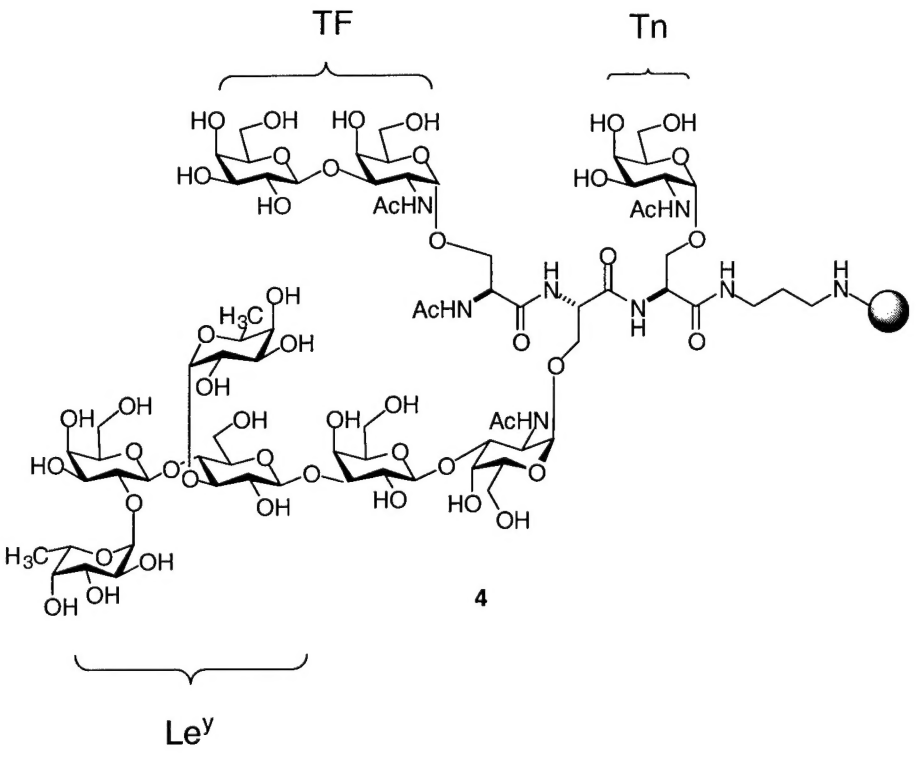


Figure 1

Polyvalent Vaccine

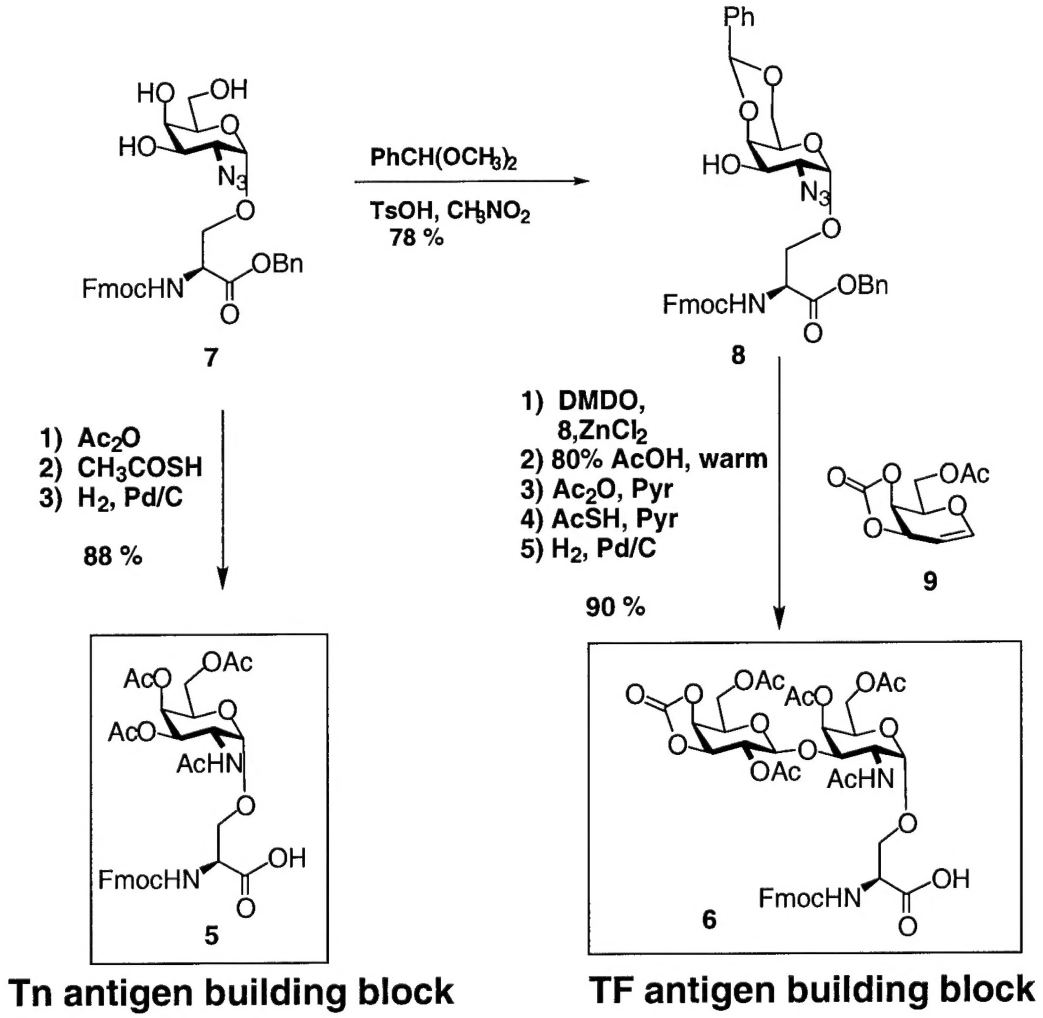


4

The initial phase of the inquiry involved the preparation of the TF (**5**) and Tn (**6**), building blocks (Scheme 2) using known methods. By utilizing a synthesis previously reported, ca. 100 g of α-*O*-linked glycan **7** was generated. The monosaccharide was converted to the Tn building block **5** in excellent overall yield (88 %, three steps) via acetate protection of the hydroxy groups followed by reductive acetylation with thiolacetic acid and hydrogenolysis. Use of the glycal assembly techniques lead to the TF building block. As shown in Scheme 2, the primary alcohol and 4-hydroxy moieties were protected via an ortho ester to give acetal **8**. The epoxide generated from glycan **9** proved to be a powerful donor in the reaction with acetal **8**. Removal of the acetal group followed by protection of the hydroxy moieties and reductive acetylation gave the

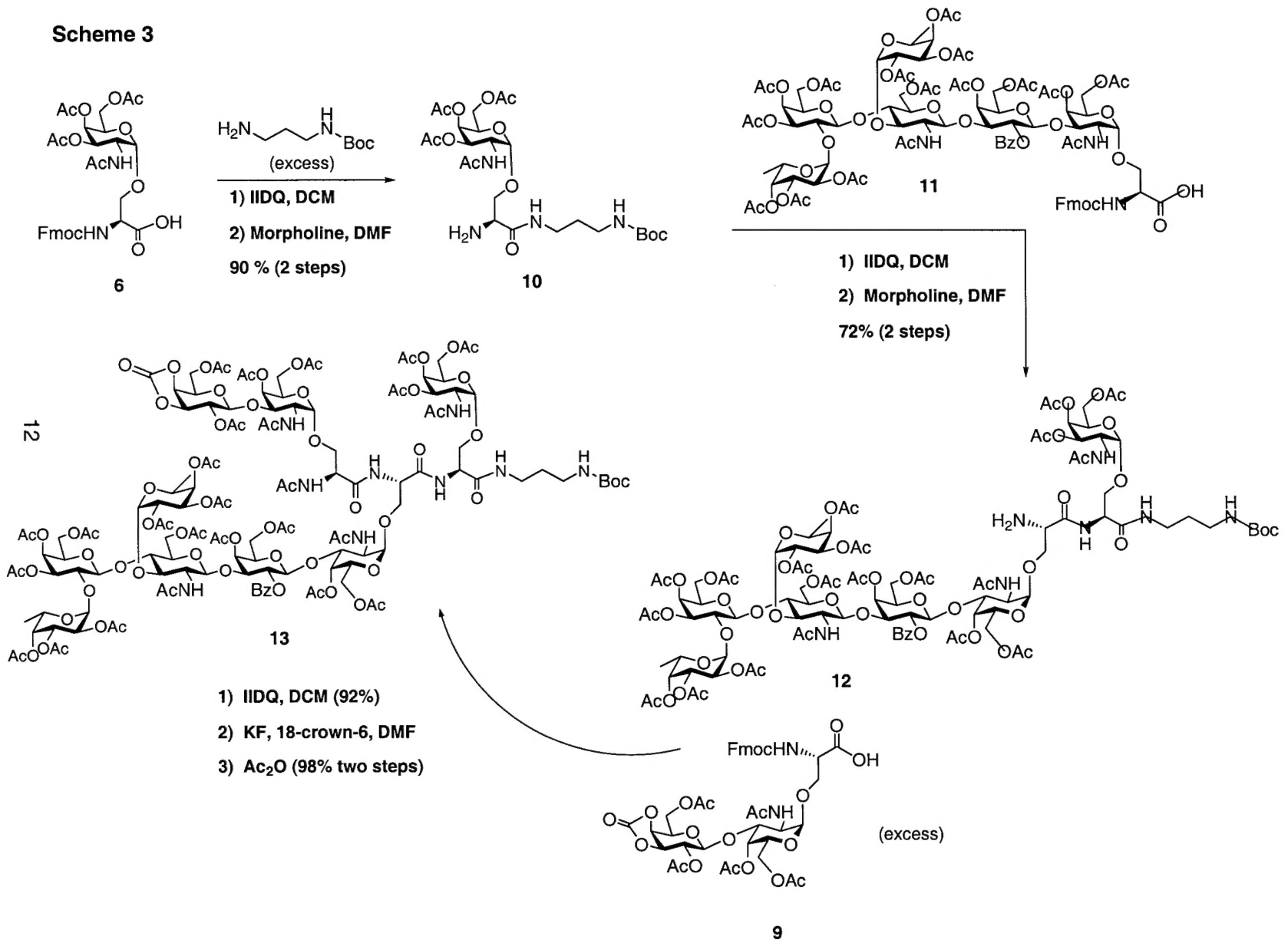
benzoyl (Bn)-protected TF building block. Finally, removal of the Bn group via hydrogenolysis afforded **6** in excellent yield (90 %; five steps).

Scheme 2



With Tn and TF building blocks in hand, the glycopeptidal backbone was assembled using cassette methodology.[9] As illustrated in Scheme 3, a diamino linker was coupled with **5** using IIDQ and Fmoc was then removed cleanly to give **10**. The coupling–deprotection sequence was repeated as **10** was reacted with the α-*O*-linked Le^y building block **11** to give **12** after Fmoc deprotection. It should be noted that the Le^y building block, which consists of a Le^y tetrasaccharide-Galactal-NAc-α–*O*-Serine core with a Galactal spacer between the Le^y tetrasaccharide and the first carbohydrate, was previously prepared using known chemistry.[9] **12** was then coupled with excess TF building block **6** in the presence of IIDQ and DCM. Finally, the protected TF–Tn–Le^y glycopeptide **13** was prepared in very good yield by removal of the Fmoc group in the presence of KF and 18-crown-6 followed by acetylation. It should be noted that previous work has shown that attempts to deprotect Fmoc with morpholine in DMF resulted in removal of the 3,4-carbonate.

Scheme 3


6
(excess)
1) IIDQ, DCM
2) Morpholine, DMF
90 % (2 steps)
10
11
1) IIDQ, DCM
2) Morpholine, DMF
72% (2 steps)
12
13
1) IIDQ, DCM (92%)
2) KF, 18-crown-6, DMF
3) Ac2O (98% two steps)
12
(excess)
9

Key Research Accomplishments:

•Efficient syntheses of the Tn, TF, and Le^y building blocks using glycal assembly.

•Syntheses of a protected glycopeptide containing TF–Tn–Le^y carbohydrates via cassette methodology.

Reportable Outcomes:

•Since this research is only in the beginning phase (ca. 5 months), no reportable outcomes pertaining directly to this project are available at this time.

Conclusions:

Glycal assembly and cassette methodoligies can be applied to the synthesis of a protected TF–Tn–Le^y glycopeptide **13**. Obviously, the future goals of this project would involve the careful removal of the acetate, carbonate, benzoyl, and Boc-groups on **13** to give the desired TF–Tn–Le^y glycopeptide **4**. Long term goals include the conjugation of **4** to KLH followed by immunological evaluation and possible clinical trials.

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